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Development of immunotherapy and gene therapy with the ability to selectively destroy tumor cells is an important goal for future breast cancer therapy. Recently in our lab, a new class of breast cancer killer cells was designed and generated by modifying lymphocytes to produce an anti-HER-2 directed toxin. A single chain antibody with high-affinity binding to the extracellular domain of HER-2 was fused with a truncated *Pseudomonas* exotoxin A gene. The transduced lymphocytes were shown to specifically kill breast cancer cells overexpressing this tumor antigen both in vitro and in a nude mouse model. The purpose of the current study is to modify and improve this novel anti-HER-2/toxin-expressing cell strategy for breast cancer therapy. Toward this goal, an efficient gene transfer system has been developed and the biological properties of the transduced lymphocytes assessed. Cytokines, such as interleukin-2 and granulocyte-macrophage colony stimulating factor, have been incorporated into the toxin expression cassette using an internal ribosome entry site in order to enhance anti-tumor activity of the transduced lymphocytes. We will attempt to increase tumor homing of the transduced lymphocytes by co-expressing cytokines and incorporate an inducible suicide mechanism for safety. This study should set the foundation for translating this novel approach for breast cancer therapy.

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FOREWORD

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Jenny Helton Hester 5/25/99
PI - Signature   Date

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Introduction

Immunotherapy with the ability to selectively destroy tumor cells has been a major goal for the treatment of breast cancer patients. The identification of tumor-associated antigens has provided one tool for selectively targeting tumor cells while allowing normal cells to remain unaffected; however, the success of various gene and immunotherapy strategies has been limited due to the inability of molecules such as antibodies or antibody conjugates to access solid tumors. HER-2 is a tumor-associated antigen which is overexpressed in many breast cancers. Our lab has developed a strategy to produce breast cancer killers cells by genetically modifying lymphocytes to produce an anti-HER-2 directed toxin. These killers cells can produce many toxin molecules at the site of the tumor thereby allowing one killer cell to kill many tumor cells. These transduced lymphocytes were able to kill breast cancer cells in vitro and in a nude mouse model. The purpose of the current study is to modify and improve this novel anti-HER-2/toxin-expressing cell strategy for breast cancer therapy. Toward this goal, an efficient gene transfer system has been developed and the biological properties of transduced lymphocytes assessed. We will attempt to increase tumor homing of the transduced lymphocytes by co-expressing cytokines and incorporate an inducible suicide mechanism for safety. This study should set the foundation for translating this novel approach for breast cancer therapy.

Body

A major goal of this project is generating a recombinant retroviral system for transducing human T lymphocytes and optimizing transduction of these retroviral vectors into human peripheral blood mononuclear cells (PBMCs). Retroviral vectors were constructed using the LNCX retroviral shuttle vector (Dr. D. Miller). A single chain antibody against HER-2 was linked to two domains of *Pseudomonas* exotoxin A (PEA) and inserted into the LNCX vector. In this vector, the anti-HER-2/toxin expression is under control of the cytomegalovirus (CMV) promoter. Human granulocyte-macrophage colony stimulating factor (GM-CSF) or human interleukin-2 (IL-2) were inserted into a bicistronic vector under control of a internal ribosome entry site (IRES) sequence in order to increase lymphocyte homing. The resulting constructs were identified by enzyme digestion and confirmed by DNA sequencing. The bicistronic vectors will coexpress the fusion toxin and cytokine.

While the retroviral vectors were being constructed and cloned, the optimal transduction protocol for transducing human peripheral blood mononuclear cells (PBMCs) using the LNCX vector containing a different expression gene was performed. Human peripheral blood cells from healthy individuals were isolated using a Ficoll-Paque density gradient. Optimal culture conditions were established by testing the concentration of human IL-2 and phytohemagluttinin (PHA) required to generate healthy rapidly dividing T cells. For transduction, a truncated nerve growth factor receptor (NGFR) was inserted into the retroviral vector under control of the viral long terminal repeat (LTR) for use as a marker to isolate transduced cells from nontransduced cells. After 48 hours of stimulation with PHA, the lymphocytes were transduced. The optimal transduction procedure requires 5×10^5 lymphocytes/ml in retroviral supernatant containing 1000 IU/ml of IL-2 and 4µg/ml of protamine sulfate. The lymphocyte/ retrovirus mixture is centrifuged at 2000rpm for 1 hour. After 24 hours incubation at 37°C, the media is replaced with fresh viral supernatant, IL-2, and protamine sulfate, and the tranduction procedure is repeated.

The transduced cells were isolated using an anti-NGFR antibody and anti-mouse IgG magnetic beads (Immunotech). The purity of the transduced lymphocytes isolated using anti-NGFR magnetic beads was greater than 95 percent. Based on the number of positively isolated cells compared to the total cell number, optimal transduction to date is approximately ten to twenty percent. The transduction procedure described above does not appear to negatively affect the lymphocytes as cells undergoing this protocol retain normal morphology, growth rate, and surface phenotype. These conditions are satisfactory to proceed with this proposal.

This study has experienced some delay as a result of the principal investigator and advisor relocation to Baylor College of Medicine, Houston, TX. The difficulties are two-fold. First, there was a significantly prolonged delay in receiving approval for the use of laboratory animals and hence a delay in grant transfer from Wake Forest to Baylor by approximately five months. Secondly, the laboratory setup and access to necessary equipment was prolonged for several weeks due to administrative decisions and problems

at Baylor College of Medicine. Despite these circumstances, the work described above does not significantly deviate from the work outlined for completion at the conclusion of 12 months by the PI's Statement of Work.

APPENDIX

Key Results
- Construction of a retroviral shuttle vector
- Optimized gene transduction procedures for human PBLs
- Evaluation of biological effects of transduction on PBLs
- Incorporation of GMCSF and IL-2 into expression vector

Reportable Outcomes
-Abstract publication for 90th annual meeting of the American Association for Cancer
  Research (AACR)

Anti-HER-2/toxin-expressing lymphocytes for breast cancer gene therapy. Hester, J.H., Yang, A-G., Chen, J., and Chen, S.-Y. *Department of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC 27157.* Development of immunotherapy and gene therapy with the ability to selectively destroy tumor cells is an important goal for future breast cancer therapy. Recently in our lab, a new class of breast cancer killer cells were designed and generated by modifying lymphocytes to produce an anti-HER-2 directed toxin. These transduced lymphocytes were shown to specifically kill breast cancer cells overexpressing this tumor antigen both in vitro and in a nude mouse model. A single chain antibody with high-affinity binding to the extracellular domain of HER-2 was fused with a truncated *Pseudomonas* exotoxin A gene. Cytokines, such as interleukin-2 and granulocyte-macrophage colony stimulating factor, have been incorporated into the toxin expression cassette and are co-expressed using an internal ribosome entry site in order to enhance anti-tumor activity of the transduced lymphocytes. The transduced lymphocytes co-express the anti-HER-2/toxin and cytokine and secrete cytokine molecules out of the cell. The effect of co-expression of cytokines with the immunotoxin will be evaluated in cell culture and animal model.